BRIEF COMMUNICATION Natural variation in levels of Immunoglobulin A in bovine milk

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INTRODUCTION

Bovine milk has been reported to contain low levels of Immunoglobulin A (IgA) in comparison to human milk (0.1 g/L vs 1.0 g/L, respectively) (Butler, 1999). However, analysis of milk collected from cows vaccinated to induce antigen-specific IgA secretion in milk (Hodgkinson, *et al.*, 2007) has demonstrated that the IgA response of individual cows is variable, with a ~10-fold range in the IgA production by individual vaccinated cows at peak lactation (E.A. Carpenter, Unpublished data). Approximately 10% of these cows are classified as "high milk IgA producers", defined by Wagter *et al.* (2000) as those cows with a milk IgA concentration greater than one standard deviation above the group mean. The opportunity exists to exploit this natural variation to generate milk products with significantly higher IgA concentrations for the infant formula and nutraceutical markets. However, the range in milk IgA concentration from non-vaccinated cows has not been reported previously. To examine this potential, milk samples from individual non-immunised cows were analysed to examine the natural variation in IgA levels in a herd of Waikato dairy cows. Further, milk was collected from individual animals over a four month period to measure the within-cow variation in milk IgA production to determine whether identifying high producing animals is repeatable during a single season.

MATERIALS AND METHODS

Animals

The 55 cows sampled in this study were pasture-fed and milked twice daily at the Ruakura No. 1 dairy farm. Milk screened to determine natural range in IgA concentration was sourced from 36 non-vaccinated cows (9 Jersey, 22 Holstein-Friesian and 5 Crossbred) in a March herd test, prior to a vaccination trial conducted during the dry period, and an additional 19 cows (8 Holstein-Friesian, 8 Crossbred and 3 Undefined breed) for which milk was collected five times over the summer months, November to February, in a subsequent year. Sampling of the 19 cows ceased in late February when the cows were placed on once daily milking due to severe drought conditions in the Waikato region. All animal treatments were approved by the Ruakura Animal Ethics Committee.

Sample collection

Morning milk samples (35 mL) were collected from individual cows via drip port during milking. The samples were kept at 4°C until they were centrifuged at 1,650 *g* for 10 minutes at 4°C. The fat layer was pushed aside and 5 mL of a skimmed milk sample was transferred to a fresh tube. Skimmed milk samples were frozen at -20°C for subsequent analysis.

Sample analysis

The IgA concentration in the milk samples was determined using a bovine IgA ELISA kit (Bethyl Laboratories, Montgomery, Texas, USA) following the manufacturer's instructions. Bovine serum was used as the reference standard in the assays. The skim milk samples were further defatted by centrifugation at 15,000 *g* for 60 minutes at 4°C following a 1/5 dilution in phosphate buffered saline. The milk whey was removed by aspiration, transferred into titre tubes, diluted to a final dilution of 1/1,000 diluent buffer (phosphate buffered saline with 0.05% Tween 20 and 1% bovine serum albumin, Sigma, St Louis, Missouri, USA) and analysed in triplicate. All the milk samples collected were analysed on a single day to remove any inter-assay variation. The intra-assay variation was determined using a quality control sample (spray-dried milk powder). The intra-assay coefficient of variation was 10.9%.

The IgA yield was calculated by multiplying the milk yield on the day of the milk sample collection by the milk IgA concentration.

Statistical analysis

Data were transformed for analysis to natural logarithms, to account for the long tail in the original distributions. The correlation of log IgA concentration with animal age in years was calculated. To estimate repeatability over the trial period, log IgA concentration was analysed in the SAS JMP package (1995), adjusting for the effects of cow age, days in milk and sample week.

RESULTS

Variation in IgA concentration in milk collected from individual cows

The endogenous total IgA concentration in milk collected from individual cows in a commercial herd in late summer varied nine-fold (0.09 – 0.81 g/L, median 0.28 g/L, n = 55). There was a positive correlation (r = 0.42) between the age of the cow and the concentration of IgA in her milk (Figure 1, P <0.05). The data also indicate an increase in IgA milk concentration after the first lactation. However, when the results were examined according to breed, the correlation between age and IgA concentration was only significant for the cross-bred animals (r = 0.75, P = 0.003, n = 13). Of the 55 individual milk samples, nine (16%) were defined as "high antibody producers", that is, their IgA concentration was greater than one standard deviation above the group mean.

Variation in milk IgA concentration over a 4 month period

The IgA concentration in milk from the 19 individual animals ranged 7-fold (0.09 - 0.62 g/L, median 0.3 g/L). However, the concentration in milk from individual animals was relatively consistent over this four month period. Two of the 19 cows (11%) were identified as "high antibody producers" on at least four of the five sampling time points (Figure 2). Milk sampling was not continued beyond 15 weeks as the cows were placed onto once-daily milking due to drought conditions in the region. Again, a positive correlation was observed the IgA yield (milk IgA concentration x milk yield) was positively correlated with age of the cow (over five sample periods, mean r = 0.68, p <0.05) (E.A. Carpenter, Unpublished data).

Repeatability estimate

Across five sample periods the repeatability estimate of milk IgA concentration for individual cows was 86% (± 5%) which demonstrated that ranking the cows for their IgA production from a single sampling would characterise a cow's IgA concentration reliably. The repeatability estimate of total IgA yield was 65% (± 11%).

DISCUSSION

Although it is generally acknowledged that bovine milk contains low levels of IgA, compared to human milk (Butler, 1999), this study demonstrates that a proportion of cows produce milk with IgA concentrations closer to that reported in human milk. Milk from individual non-vaccinated dairy cows demonstrated an approximate 9-fold range in IgA concentration. Variation to at least this extent has been reported in studies of IgA concentration in human milk (Weaver, *et al.* 1988). There appears to be a tendency for increased IgA concentration in milk of cows with increasing age, particularly with cross-bred animals. The total IgA yield also increased with increasing age. The results also indicate that milk IgA concentration increased after the first lactation. However, because this study was conducted using a commercial herd with external factors influencing decisions on cull rate, such as milk volume, mastitis history, and even temperament, these data must be treated with caution.

FIGURE 1: Concentration of total IgA (g/L) in milk samples from individual dairy cows of varying age from two commercial herds at late lactation (February-March). Three breeds were represented: ▲ Jersey (n = 9), □ Holstein-Friesian (n = 30), ● Cross-bred (Jersey x Holstein-Friesian) (n = 13). Standard deviation of milk IgA concentration is shown with a vertical bar.

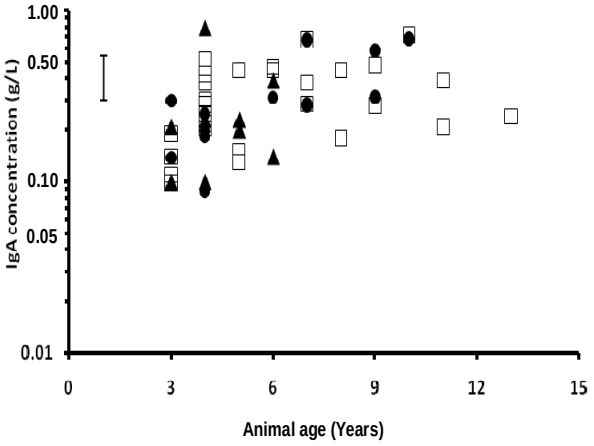
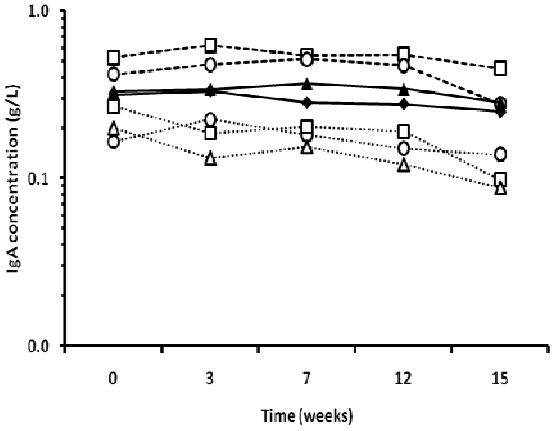


FIGURE 2: Concentration of total IgA (g/L) in milk collected from individual cows over a 15 week period (November-February). For clarity, data from only 7 of the 19 animals are presented. Dashed lines represent the two cows with [IgA] >mean + one standard deviation unit on at least four time points; Solid lines represent cows with mean [IgA]; Dotted lines represent three animals with [IgA] <mean – one standard deviation unit on at least four time points.



Despite the wide variation in IgA concentration in milk collected from different animals, the individual cow milk IgA concentration was relatively constant over the four month study period. Using these data, repeatability estimates demonstrated it was possible to rank cows for their IgA production from a single milk sampling. Three cows were identified as "high IgA producers" at the first sampling (Week 0). Collection of milk from these animals separately to the remainder of the herd over the following 15 weeks could have led to a ~ 45% increase in milk IgA concentration, compared to the mean IgA concentration from all cows in the original herd, with no further manipulation required. Conversely, removing four animals identified as low IgA producers with a milk IgA concentration less than the herd mean minus one standard deviation, could have led to a 10% increase in herd milk IgA concentration.

This study demonstrates that generation of milk with enhanced concentrations of natural IgA is potentially feasible for producers with the appropriate cow numbers and dairy facilities. Increased IgA concentrations in milk will generate milk powders with enhanced levels of IgA, which will be attractive for infant formula and nutraceutical markets.

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